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Physiological variations between female West African dwarf Goat and Sheep

Uwaeziozi, U.C.[1], Jimoh, O. A.[2], Ihejirika, U.G. D.[3], Jack, A. A.[4], Adenekan, O. O.[5]
1, 3-5 Department of Animal Science, University of Ibadan, Nigeria
2 Agricultural Technology Department Federal Polytechnic Ado-Ekiti, Ekiti State Nigeria
Uche19822000@gmail.com, abubarkarjimoh2011@gmail.com, smile4me001@yahoo.com, akanjack01@yahoo.com, adenekan.olusola@yahoo.com

ABSTRACT:
Haematological, Serum Biochemistry profile and Oxidative stress indicators of female West African Dwarf Goat and Sheep respectively were investigated. Sixteen apparently healthy each of ewes and does of the West African breed were selected from a flock and housed separately under a semi- intensive system. Fastened blood samples were collected from their jugular vein to obtain specie variations in some haematological, serum biochemistry and oxidative stress indicators. Blood was collected to EDTA and plain bottles for haematology and serum biochemistry respectively using standard procedures. Data were subjected to T-test. The RBC, MCH, MCHC, Monocytes and Eosinophils showed significant differences (P<0.05) between species, where MCH and MCHC of the Ewes (0.60±0.08 and 20.52±0.41 respectively) were significantly (P<0.05) higher than those of the Does (0.39±0.20 and 18.08±3.63 respectively). The Glucose and Albumin levels were significantly lower in the ewes compared to the does. The total antioxidant activity and catalase level of the ewes were significantly (p<0.05) higher than those of the does while lipid peroxidation and superoxide dismutase level of the does were significantly (P<0.05) higher than those of the ewes. In conclusion, it can be deduced that so much similarities exists between species (does and ewes). Ewes have better oxidative stability than does and are less prone to oxidative stress.

Keywords: Haematology, Serum biochemistry, oxidative stress, West African dwarf, Doe, Ewe.

INTRODUCTION

The global sheep stock recorded by UN food and Agriculture Organisation in 2008 revealed about 33.9 million sheep in Nigeria and has grown to about 39 million by 2013 [1].

Man and animals are exposed to a large number of biological and environmental factors like alterations in feed and husbandry practices, climatic variables, transportation, regrouping, the therapeutic and prophylactic activities, various stressors, and so forth. Oxidative stress occurs when the homeostatic processes fail and free radical generation is much beyond the capacity of the body's defences, thus promoting cellular injury and tissue damage. This damage may involve DNA and protein content of the cells with lipid peroxidation of cellular membranes, calcium influx, and mitochondrial swelling and lysis [2,3,4]. In nature there are thousands of compounds possessing antioxidant properties. There are both fat-soluble (vitamin E and carotenoids, etc.) and water-soluble (ascorbic acid, glutathione, bilirubin, etc.), they can be synthesized in the body (ascorbic acid, glutathione) or are delivered with food/feed (vitamin E, carotenoids, Se etc.). The antioxidant enzymes that are synthesised in the body require metal co-factors. For example, selenium in the form of selenocysteine is at the active site of several families of enzymes such as the glutathione peroxidases (GSH-Px) and thioredoxin reductase (TR). Zinc, copper and manganese are integral parts of another antioxidant enzyme family called superoxide dismutases (SOD); and iron is an essential part of the antioxidant enzyme called catalase. Only when these metals are delivered with the diet in sufficient amounts can the body synthesize the antioxidant enzymes. In contrast, deficiency of those elements causes oxidative stress and allows damage to biological molecules and membranes. It is important to realize that all antioxidants in the body function in concert to provide antioxidant defence.

The immune system of the animal is based on natural and adaptive immunity. The natural immunity is dependent on the efficient function of phagocytic cells namely neutrophils and macrophages which use free radicals as a weapon to kill pathogen, however, when in escape from phagosome the same free radicals become dangerous and can damage all sort of biological molecule compromising phagocyte function and damaging adaptive immunity. Phagocytes also produce so called communication molecules (eicosanoids, cytokines, etc.) which are used for effective communications between various immune cells. Adaptive immunity is based on

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activity of B- and T-lymphocytes which are producing antibodies. As one could realise that communication between immune cells is a crucial factor of immunocompetence. If we imagine that immune system is an army fighting against invaders (microorganisms, viruses, etc) then we would expect them to have something like mobile phones to receive and send signals to each other. Remarkably enough, major immune cells (macrophages, neutrophyls, T- and B-lymphocytes) have on their surface something like "mobile phones" called receptors. Those receptors are extremely sensitive to communicating molecules, but they are also sensitive to free radicals and can be easily damaged. In such a situation without proper communication all those huge armies of immune cells would become useless. They also can start fighting each other as well and eventually destroying immunocompetence. If we imagine that immune cells are soldiers using chemical weapon to kill enemy, then special ammunition protecting them from their own weapon would be crucial for effective battle. In the case of immune cells such ammunition is represented by natural antioxidants with Se-GSH-Px being a major defence. Based on the presented model it is clear that antioxidant defence is a crucial factor of immune defence in the body [5]. Studies have also revealed that oxidative stress caused by transportation in hot humid tropical condition can bring about haematological derangements [6].

The West African Dwarf goats and sheep are found in the eco – zone infested with tse-tse fly and the dwarf goats thrive well [7], thereby satisfying a part of the meat requirement in this region These advantages recorded in both sheep and WAD goats presents great potentials to mitigate the problems of protein malnutrition in those regions; therefore, having a knowledge of the variations of haematological parameters, serum biochemical parameters and oxidative stress status between these species could help in realistic evaluation of the management practices, nutrition and diagnosis of health condition.

MATERIALS AND METHODS

Data collection

Blood samples were collected from the jugular vein of 16 apparently healthy females each of sheep and goat of the West African dwarf breed. The Ewes and Does had average weights of 12.08 ± 3.26kg and 12.15 ± 1.51kg respectively. The experiment was carried out on a completely randomized design. Ten (10ml) of blood samples were collected and 5ml out of it was emptied into Ethylene Diamine Tetra Acetic Acid (EDTA) bottle, for haematological evaluation and the remaining 5ml was used to analyse for biochemical parameters in a bottle without anticoagulant. The blood samples were transferred to the laboratory for analysis within 12 hours of collection.

Haematological parameters:

Packed cell volume (PCV), Haemoglobin (Hb), Red Blood cell(RBC), White blood cell (WBC)$X10^3$, Lymphocytes $X10^3$, Neutrophils $X10^3$, Monocytes and Eosinophils were analysed as described by [8].

Mean cell volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) were calculated using standard formula.

SERUM BIOCHEMISTRTY PARAMETERS

Serum separation was done by centrifuging blood samples at 3000rpm for 5 minutes and within 45 minutes of blood collection. Serum obtained was further analysed for the following indices: Glucose, Total Protein, Albumin, High density lipoprotein, Cholesterol, Triglyceride, Alkaline Phosphatase (ALP), Creatinine, Alanine amino transferase, Aspartate amino transferase, Urea. Serum biochemical indices were analysed spectrophotometrically by using commercially available diagnostic kits (RANDOX ® Test Kits).

OXIDATIVE STRESS INDICATORS

Serum total antioxidant capacity activities was carried out according to [9]. Superoxide dismutase (SOD) was estimated by the method of [10] adopted by Soon and Tan, [11] and Catalase was estimated by [12] method. Serum lipid peroxidation was determined using thiobarbituric acid assay according to [13].

STATISTICAL ANALYSIS

Data were analysed using the one-way ANOVA of [14] procedure and Duncan multiple range test

option of the same statistical software was used to separate the treatment means.

RESULTS

The results of the haematological variations between female West African Dwarf goats and sheep are shown in table 1. The RBC count of the Does (11.70±1.04 $x10^6/mm^3$) were significantly (P< 0.05) higher than those of the Ewes (11.62±0.08 $x10^6/mm^3$) The monocytes and the eosinophils' count of the Does (2.94± 1.52 $x10^3/mm^3$ and 2.57±1.55 $x10^3/mm^3$ respectively) were significantly (P< 0.05) higher than those of the ewes (1.30± 0.60 $x10^3/mm^3$ and 2.51±1.58 $x10^3/mm^3$ respectively). The Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) of the Ewes (0.60±0.08pg and 20.52±0.41% respectively) were significantly (P<0.05) higher than those of the Does (0.39±0.20pg and 18.08±3.63% respectively). Packed Cell Volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), White blood cells (WBC), Lymphocytes and Neutrophils' values of the species were not different from one another.

The results of variations in the serum biochemistry profile of female West African Dwarf goats and sheep are shown in table 2. Glucose(mg/ml) and albumin (g/dl) concentrations of the Does (5.52±1.91 and 9.79±5.21, respectively) were significantly (P<0.05) higher than those of the ewes (1.79±0.87 and 3.44±2.66, respectively). The total protein, high density lipoprotein, cholesterol, triglyceride, alkaline phosphatase, creatinine, Alanine amino transferase, Aspartate amino transferase and urea values of the species were not different from one another

The result of the variation in oxidative stress indicators of female West African Dwarf goats and sheep are as shown in table 3. Total antioxidant and catalase activity were significantly (P<0.05) higher in the Ewes (8.42±1.82 and 24.24±6.67, respectively) compared to those of the Does (8.37±2.67 and 8.04±1.78, respectively). Lipid peroxidation was significantly (P<0.05) higher in the Does (3.76±2.80) compared to that of the Ewes (1.77±1.36). Higher level (1.25±0.61) of superoxide dismutase was observed in the Does and was significantly different (P<0.05) compared to that of the Ewes (0.49±0.10).

DISCUSSION

Blood which is a vital special circulatory tissue is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis [19]. The PCV and haemoglobin level for both species were not significantly different from each other but numerically higher levels of PCV (0.34±0.08) and haemoglobin (7.01±1.59) was recorded for ewes compared to those of the does (0.24±0.10 and 4.58±2.35, respectively). This result is affirmed in the level of red blood cell recorded for both species which were significantly different from each other. PCV which is packed cell volume accounts for the volume of red blood cells in a measure of blood while haemoglobin is the substance in red blood cell capable of transporting oxygen from the lungs to the body tissues. This result further implies that the volume of PCV and haemoglobin in the blood is dependent on the quantity of red blood cells in the blood. RBC in this study was significantly higher in the Does (11.70±1.04) compared to that of the Ewes which had 11.62±1.62. The results of the Does and the Ewes corresponded to the values reported by [15] (10.8±1.0) for WAD does and [16] for adults Ewes of the temperate breed (11.60±0.67). Differences in the values for RBC in WAD ewes compared to other arid breeds like the Yankassa (9.31±0.78) and Ouda breed (11.90±1.11) has been observed as recorded by [17]. This should be as a result of breed differences which has also been reported by [18] on Ethiopian sheep breeds. Red blood cell is involved in the transport of oxygen and carbon dioxide in the body [19]. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs [20, 21,19). This result implies better oxygen circulation in the does than the ewes which suggests that ewes (sheep) should be managed in a well ventilated environment.

The MCH and MCHC of the WAD Ewes (0.6±0.08 and 20.52±0.41, respectively) were higher than those of the WAD Does (0.39±0.20 and 18.08±3.63). These findings were lower than the reports of [16], in temperate breeds and the MCHC of WAD Does were also lower than the

reports of [15] on WAD does (33.9±0.2). Although the RBC result corresponded with some literatures [15,16], MCH and MCHC seemed to be lower than what is obtained in these literatures. A normal RBC count but with low MCH (Average amount of Hb in a RBC) and MCHC (Average amount of Hb compared to the size of the RBC) may indicate an impending anaemic condition caused by Iron deficiency. An Iron supplementation or diet improvement may balance this discrepancy. The higher MCH and MCHC of ewes compared to the does may imply differences in size of RBCs between the species. Does may be having smaller sizes of RBC in a volume of blood compared to the ewes which further indicates that high volume of Hb may be accommodated meaning increased O_2 circulation. But in this case, a lower MCH and MCHC was recorded for the specie with higher RBC count indicating that the Does' nutrition should be fortified with more iron containing feed materials and Vitamin B9 (Folic acid) to avert their anaemia prone body system. Vitamin B9 works with vitamins B6 and B12 and other nutrients to control blood levels of amino acid- which are constituents of proteins of which haemoglobin in one.

According to Research Animal Resources [22] the reference value for sheep eosinophils ranges between 0-10% while monocytes ranges between 0-6% also the hematological values of normal ewe as documented by [23] which reveals eosinophils within the range of 3.60-12.4% and monocytes within the range of 0.72-3.28% corresponds to the values recorded for WAD ewes in this study (2.51±1.58% for eosinophils and 1.30±0.60% for monocytes). The values of eosinophils and monocytes for WAD does in this study is in line with the reports of [23] for the range values for a normal doe (2.46-4.54% for eosinophils and 1.56-2.44% for monocytes). Eosinophils are a type of white blood cell that play an important role in the body's response to allergic reactions and infections with parasites while monocytes are white blood cells that help other white blood cells to remove dead or damaged tissues. Apart from specie difference which could be a factor affecting the difference in values of eosinophils and monocytes in does and ewes, the hardy and resilient nature of goats could also be responsible in building more of these cells to strengthen the defence system of their body, also the higher value of eosinophils and monocytes in the does compared to the ewes may present that the does has better defence system against parasitic and allergic infections and also in sanitizing the immune system.

The glucose (mg/ml) level in the does (5.52±1.91) were higher than that of the ewes (1.79±0.87) and these values were quite lower than the results stated for WAD does (25.1±4.6mg/dl) by [24] and for a normal ewe (80.8mg/dl) as reported by [25]. The result of the WAD ewes was also lower than those of Yankasa, Ouda and Balami ewes (60mg/dl, 50mg/dl and 52mg/dl, respectively) which are breeds also predominant in the sahel savannah regions of Nigeria as stated by [17]. The differences in values between WAD and these three breeds could be attributed to breed differences, although there was a marked difference in the results for WAD does compared to the results recorded by [24], this could be as a result of environmental conditions, age and activity level of the animals. The low level of glucose in this study compared to those obtained from literature could also be as result of level of vitamin B complex in their diet which may be low which could also account for the low levels of MCH and MCHC; this is because all B vitamins help the body convert food (carbohydrates) into fuel (glucose), which is used to produce energy, also vitamin B12 works closely with vitamin B9, also called folic acid, to help make red blood cells and to help iron work better in the body.

There was apparently higher glucose level in WAD does than was observed in the WAD ewes. Glucose primary function is to provide energy for physiological processes such as respiration, muscle contraction and relaxation, heart rhythm and the regulation of body temperature [26]. The mammalian brain depends upon glucose as its main source of energy [27]. The apparently high glucose level in the does (goats) may be reflected in high physical activity levels in goats compared to sheep

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Table 1: Haematological Variations between female West African Dwarf Goat and Sheep

Parameters	Doe±SD	Ewe±SD	P value	Significance
Packed cell volume(L)	0.24±0.10	0.34±0.08	0.54	NS
Haemoglobin (g/dl)	4.58±2.35	7.01±1.59	0.22	NS
Red Blood cell ($x10^6/mm^3$)	11.70±1.04[a]	11.62±1.62[b]	0.03	SIG
Mean cell volume (fl)	20.26±8.42	29.17±3.86	0.23	NS
Mean cell haemoglobin (pg)	0.39±0.20[b]	0.60±0.08[a]	0.01	SIG
Mean cell haemoglobin concentration (%)	18.08±3.63[b]	20.52±0.41[a]	0.00	SIG
White blood cell ($x10^3/mm^3$)	10.46±2.33	4.93±2.20	0.88	NS
Lymphocytes ($x10^3/mm^3$)	6.99±1.88	2.82±1.35	0.25	NS
Neutrophils ($x10^3/mm^3$)	3.05±0.98	1.43±0.94	0.62	NS
Monocytes (%)	2.94±1.52[a]	1.30±0.60[b]	0.01	SIG
Eosinophils (%)	2.57±1.55[a]	2.51±1.58[b]	0.03	SIG

SD- Standard Deviation; P value- probability value; SIG- Significant difference; NS- Not Significantly different

Table 2: Variations in the Serum Biochemistry profile of female West African Dwarf Goat and Sheep

Parameters	Doe±SD	Ewe±SD	P value	Significance
Glucose (mg/ml)	5.52±1.91[a]	1.79±0.87[b]	0.01	SIG
Total protein (g/dl)	102.69±17.46	32.91±11.92	0.12	NS
Albumin (g/dl)	9.79±5.21[a]	3.44±2.66[b]	0.06	SIG
High density lipoprotein (mmol/l)	2.24±0.81	1.94±0.57	0.22	NS
Cholesterol (mmol/l)	1.24±0.79	1.03±0.59	0.47	NS
Triglyceride (mmol/l)	0.75±0.28	.52±0.17	0.25	NS
Alkaline phosphatase(IU/L)	1594±246.85	1216.62±309.37	0.61	NS
Creatinine (Mmol/l)	189.24±86.43	163.0±187.16	0.146	NS
Alanine amino transferase (IU/L)	6.55±2.53	5.54±2.79	0.80	NS
Aspartate amino transferase(IU/L)	11.42±1.69	14.50±4.88	0.116	NS
Urea (mg/dl)	131.64±21.46	193.38±20.58	0.48	NS

SD- Standard Deviation; P value- probability value; SIG- Significant difference; NS- Not Significantly different

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Table 3: Variation in Oxidative stress indicators of female West African Dwarf Goat and Sheep

Parameters	Doe±SD	Ewe±SD	P value	Significance
Total antioxidant activity (mmol/litre)	8.37±2.67[b]	8.42±1.83[a]	0.00	SIG
Catalase (mmoles of H_2O_2 consumed/min/mgprotein)	8.04±1.78[b]	24.24±6.67[a]	0.00	SIG
Lipid peroxidation (x103TBARS/mgprotein)	3.76±2.80[a]	1.77±1.36[b]	0.01	SIG
Superoxide dismutase(SOD) (U/g)	1.25±0.61[a]	0.49±0.10[b]	0.047	SIG

SD- Standard Deviation; P value- probability value; SIG- Significant difference

The Albumin level (g/dl) of the does 9.79±5.21 were higher than that of the ewes 3.44±2.66. The Albumin results for both Doe and Ewe fell within the range of normal doe (2.45-4.35g/dl) and normal ewe (2.70-4.55g/dl) as documented by [28]. Serum albumin being a protein functions to bind water, cations (such as Ca2+, Na+ and K+), fatty acids, hormones, bilirubin, thyroxin and regulates Oncotic (water in the circulatory system) pressure of the blood. This result may imply greater metabolic activities and nutrient circulation in does compared to the ewes. This further indicates that the does may utilize nutrients from feed faster hence promoting general body growth.

Total antioxidant activity and Serum Catalase in the ewes were significantly higher ($P<0.05$) than that of the does. The high antioxidant activity could be attributed to its higher catalase activity which indicates higher scavenging of hydrogen peroxides. Periods of increased metabolic activity implies an increase in the production of free radicals, which are produced in the mitochondria as a normal by-product of cellular respiration at the electron transport chain reaction [29]. Several studies suggest that oxidative stress increases the susceptibility of animals to diseases [30]. Lipids are polyunsaturated and are prone to oxidation. Lipids are one of the most susceptible substrates to free radicals' damage and biomarkers of Lipid peroxidation are considered the best indicators of oxidative stress [31]. Over production of reactive species results in oxidative stress, these reactive species of oxygen – nitrogen derived radicals reduce antioxidant capacity, causing an imbalance that result in the attack of cellular components especially lipids [32]. Superoxide dismutase is the first line of defense in which superoxide are dismutated by SOD to hydrogen peroxide which is further converted to water and other lesser species by Catalase. The role of Superoxide dismutase is to accelerate the dismutation of the toxic superoxide produced during oxidative energy processes to hydrogen peroxide and molecular oxygen [33]. Lipid peroxidation and SOD were significantly ($p<0.05$) higher in the does compared to the ewes, the higher lipid peroxidation is an indication of oxidative stress which led to the increase in SOD to counter toxic superoxides which may be produced as a result. Higher physical and metabolic activities of the goats over the sheep may be exposing the does to higher oxidative stress more than the ewes hence more susceptible to diseases.

CONCLUSION

In conclusion, it can be deduced that so much similarities exists between species (does and ewes), but differences exist in RBC, MCH, MCHC, monocytes, eosinophils, glucose and albumin levels. These differences create the impression that they have different immune responses a well as energy capacity. The immune system defence against parasitic infection and allergic reaction are stronger in the does compared to the ewes while the glucose and albumin levels reveals a greater metabolic activities and nutrient circulation in does, and further indicates that the does may utilize nutrients from feed faster hence promoting general body growth. This result indicates that efforts should be made to ensure

that feeds of does should be supplemented with iron and vitamin B complex to shield against anaemia as indicated by low MCH and MCHC.

The differences in oxidative stress status indicates that ewes have superior antioxidant defense over the does and thus have lower susceptibility to oxidative stress and by extension diseases compared to the does. Efforts to improve antioxidant protection of does during high metabolic periods in management like pregnancy periods should be made. In general, proper and exclusive management practices should be adopted for ewes and does respectively so as to have a healthy flock.

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Our ref..... Your ref..... Date: 6th June, 2017.

The Editor,

SUBMISSION DECLARATION

I humbly declare that this research titled: "***OXIDATIVE STRESS AND METABOLIC INDICES OF WEST AFRICAN DWARF BREEDS OF SHEEP AND GOAT IN NIGERIA***" was undertaken with approval from institutional ethics committee of the Department of Agricultural Technology, Federal Polytechnic, Ado-Ekiti.

The institutional and national standards for the care and use of animals for research in the Research Policy Handbook of the Federal Polytechnic, Ado-Ekiti were followed and appropriate measures were taken to minimize pain or discomfort on the animals.

Yours sincerely,

M.O. Akinola, Ph.D
Head of Department